Antiarrhythmic effects of the highly selective late sodium channel current blocker GS-458967

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Abstract

BACKGROUND—Previous studies have shown that late sodium channel current (I_{Na}) blockers such as ranolazine can exert antiarrhythmic effects by suppressing early and delayed after-depolarization (EAD and DAD)-induced triggered activity.

OBJECTIVE—To evaluate the electrophysiological properties of GS-458967 (GS967), a potent and highly selective late I_{Na} blocker, in canine Purkinje fibers (PFs) and pulmonary vein (PV) and superior vena cava (SVC) sleeve preparations.

METHODS—Transmembrane action potentials were recorded from canine PFs and PV and SVC sleeve preparations by using standard microelectrode techniques. The rapidly activating delayed rectifier potassium channel current blocker E-4031 (2.5–5 μM) and the late I_{Na} agonist ATX-II (10 nM) were used to induce EADs in PFs. Isoproterenol (1 μM), high calcium ($[Ca^{2+}]_o = 5.4$ mM), or their combination was used to induce DADs and triggered activity.

RESULTS—In PFs, GS967 (10–300 nM) caused a significant concentration-dependent reduction in action potential duration without altering the maximum rate of rise of the action potential upstroke, action potential amplitude, or resting membrane potential at any rate studied (basic cycle lengths of 1000, 500, and 300 ms) or concentration evaluated (n = 5; $P < .05$). GS967 (30–100 nM) abolished EADs and EAD-induced triggered activity elicited in PFs by exposure to E-4031 (n = 4) or ATX-II (n = 4). In addition, GS967 reduced or abolished DADs and suppressed DAD-induced triggered activity elicited in PFs (n = 4) and PV (n = 4) and SVC (n = 3) sleeve preparations by exposure to isoproterenol, high calcium, or their combination.

CONCLUSIONS—Our data suggest that the selective inhibition of late I_{Na} with GS967 can exert antiarrhythmic effects by suppressing EAD- and DAD-mediated extrasystolic activity in PFs and PV and SVC sleeve preparations.

Keywords

Antiarrhythmic drugs; Electrophysiology; Pharmacology; Late sodium current; Ranolazine

Introduction

Purkinje fibers (PFs) have been shown to develop marked action potential prolongation as well as early afterdepolarization (EAD)-induced triggered activity following exposure to agents that slow ventricular repolarization[1,2] or promote delayed after-depolarization

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(DAD)-induced triggered activity under conditions of calcium overload or beta-adrenergic stimulation.[3,4]

Pulmonary veins (PVs) have been shown to be the most common ectopic sites capable of initiating atrial fibrillation (AF).[5] The superior vena cava (SVC) is the main non-PV site of origin of extrasystoles capable of triggering AF.[6] Previous studies have shown that late phase 3 EAD- and DAD-induced triggered activity can be easily elicited in PV and SVC sleeve preparations by parasympathetic and/or sympathetic stimulation.[7,8] In addition to PFs, PV, and SVC, epicardial ventricular muscle cells have been reported to develop EADs and triggered activity in aged rat hearts exposed to oxidative stress.[9] In the last several years, a number of studies have shown that the inhibition of late sodium channel current (I_{Na}) with drugs such as ranolazine, flecainide, and mexiletine exerts antiarrhythmic effects by suppressing EAD- and DAD-induced triggered activity in experimental models (see Reference[10] for review). However, none of these drugs are sufficiently selective inhibitors of late I_{Na} to provide definitive evidence of the role of this current in the genesis of arrhythmias in various cardiac tissues.

A recent study showed that the late I_{Na} blocker GS-458967 (GS967) can exert antiarrhythmic effects in rabbit ventricular myocytes, rabbit isolated hearts, and anesthetized rabbits.[11] EADs and EAD-induced triggered activity were observed in isolated myocytes, and EAD-dependent extra-systoles were observed in isolated rabbit hearts.

The present study was designed to evaluate the electro-physiological and antiarrhythmic properties of GS967 previously described in the isolated rabbit heart in diverse cardiac preparations such as canine PFs and PV and SVC sleeve preparations known to be involved in the genesis of extrasystoles responsible for the development of major cardiac arrhythmias including AF and ventricular tachycardia/ ventricular fibrillation. Preliminary data have been reported in abstract form.[12]

Methods

This investigation conforms to the *Guide for Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Animal Care and Use Committee of the Masonic Medical Research Laboratory.

Adult mongrel dogs weighing 20–35 kg were anticoagulated with heparin (180 IU/kg) and anesthetized with sodium pentobarbital (35 mg/kg, intravenous). The chest was opened via a left thoracotomy, and the heart was excised and placed in a cold cardioplegic solution ($[K^+]_0$ = 12 mM; 4°C).

PFs and PV and SVC sleeve preparations

PFs (false tendons) were isolated from left or right canine ventricles; PV and SVC sleeve preparations (approximately 2.0 × 1.5 cm) were isolated from the left and right canine atria, respectively. The thickness of the PV and SVC preparations was approximately 2 mm. The preparations were placed in a small tissue bath and superfused with Tyrode's solution of the following composition: 129 mM NaCl, 4 mM KCl, 0.9 mM NaH_2PO_4, 20 mM $NaHCO_3$, 1.8 mM $CaCl_2$, 0.5 mM $MgSO_4$, 5.5 mM glucose, buffered with 95% O_2/5% CO_2 (35 ± 0.5°C). PFs and PV and SVC sleeve preparations were stimulated at a basic cycle length (BCL) of 1000 ms during the equilibration period (1 hour) using electrical stimuli (1–3 ms duration; 2.5 times diastolic threshold intensity) delivered through silver bipolar electrodes insulated except at the tips. Transmembrane potentials were recorded by using glass microelectrodes filled with 3 M KCl (10–20 M direct current resistance) connected to a

high input-impedance amplification system (Model KS-700, World Precision Instruments, New Haven, CT). In PFs, action potential duration at 90% repolarization (APD_{90}), maximum rate of rise of the action potential upstroke (V_{max}), action potential amplitude (APA), and MDP were evaluated before and after exposure to GS967. Transmembrane action potentials (APs) were recorded at a sampling rate of 41 kHz.

Induction of arrhythmias

The rapidly activating delayed rectifier potassium channel current (I_{Kr}) blocker E-4031 (2.5–5 μM) and the late I_{Na} agonist ATX-II (10 nM) were used to induce EADs and triggered activity in canine PFs. Isoproterenol (1 μM), high calcium ($[Ca^{2+}]_o = 5.4$ mM), or their combination was used to induce DADs and triggered activity in canine PFs and PV and SVC sleeve preparations. This arrhythmia model has previously been shown to generate DAD-induced triggered activity in 100% PV sleeve preparations.[7]

Drugs

GS967 (Gilead, Foster City, CA) was dissolved in distilled water to form a stock solution of 100 μM and used at a final concentration of 10–300 nM. Isoproterenol (Sigma-Aldrich, St Louis, MO) was dissolved in distilled water to form a stock solution of 5 mM and used at a final concentration of 1 μM. E-4031l (Sigma-Aldrich) was dissolved in distilled water to form a stock solution of 5 mM and used at a final concentration of 2.5–5 μM. ATX-II (Sigma-Aldrich) was dissolved in distilled water to form a stock solution of 200 nM and used at a final concentration of 10 nM.

Statistical analysis

The statistical analysis of the electrophysiological effects of GS967 in canine PFs was performed by using 1-way repeated measures analysis of variance followed by the Bonferroni test. The statistical analysis of incidence of EADs was assessed by using the Fisher exact test. Mean values were considered to be significantly different at $P < .05$. All data are reported as mean $\pm$ SD.

Results

We determined the viability and stability of superfused PFs and PV and SVC sleeve preparations by superfusing them with normal Tyrode's solution and continuously recording the electrical activity for a period up to 120 minutes. No significant changes in AP morphology were observed over the 2-hour period.

Electrophysiological effects of GS967 in canine PFs

In one series of experiments, we determined the effect of GS967 on the electrophysiological characteristics of canine PFs isolated from both ventricles of normal dogs. As illustrated in Figure 1A, GS967 caused a marked concentration-dependent reduction of APD without altering APA or MDP. Data summarizing the effects of GS967 on APD_{90} and V_{max} are shown in Figure 1B. APD_{90} was significantly reduced at BCLs of 1000, 500, and 300 ms and concentrations of 30, 100, and 300 nM. At a concentration of 100 nM, APD_{90} was reduced from 347 ± 83 to 281 ± 65 ms at a BCL of 1000 ms and from 245 ± 53 to 205 ± 46 ms at a BCL of 300 ms. No significant changes in V_{max}, APA, or MDP were observed at any BCL or concentration studied. At a BCL of 1000 ms, APA and MDP were 122.4 ± 8 and -85.2 ± 5 mV, respectively, under control conditions and 121.9 ± 7 and -85.4 ± 5 following GS967 (100 nM). At a BCL of 300 ms, APA and MDP were 121.8 ± 7 and -85.1 ± 5 mV, respectively, under control conditions and 120.7 ± 7 and -84.8 ± 5 mV following GS967 (100 nM).

Effects of GS967 on EADs and triggered activity induced in canine PFs

In another series of experiments, we determined the effects of GS967 on EADs and triggered activity induced by the I_{Kr} blocker E-4031 (2.5–5 μM) and the late I_{Na} agonist ATX-II (10 nM). Figure 2 illustrates a representative example of the effect of GS967 to suppress EADs and triggered activity elicited by exposure to E-4031 in a canine PF. At a BCL of 3500 ms, E-4031 (2.5 μM) induced EADs and triggered activity (Figure 2A). The addition of GS967 (30 nM) suppressed all EADs and triggered activity (Figure 2B). The washout of E-4031 restored EAD and triggered activity (Figure 2C). Figure 3 shows another example of the concentration and rate dependence of the effect of GS967 to reduce APD and suppress EAD-induced triggered activity elicited by exposure to E-4031. Figure 2D illustrates the incidence of EAD-induced triggered activity in 5 PFs exposed to E-4031 (2.5–5 μM) alone or together with 30 or 100 nM GS967. The incidences of EAD-induced triggered activity were 4 of 4 (100%), 2 of 5 (40%), and 0 of 5 (0%) PFs following E-4031, 30 nM GS967, and 100 nM GS967, respectively (P = .029, E-4031 vs 100 nM GS967).

Figures 4 and 5 illustrate the effects of GS967 to suppress EADs and EAD-induced triggered activity elicited by exposure to ATX-II in a canine PF. As shown in Figure 4, ATX-II (10 nM) elicited EADs and EAD-induced triggered activity at BCLs of 2000, 2500, 3500, 4500, and 8000 ms (Figure 4A). The addition of GS967 (30 nM) abolished all EADs and triggered activity (Figure 4B). Figure 5 illustrates the concentration and rate dependence of the effect of GS967 to suppress EADs and EAD-induced triggered activity elicited by exposure to ATX-II in a canine PF. ATX-II (10 nM) induced EADs and triggered activity at BCLs of 2000 and 3000 ms and depressed spontaneous firing (Figure 5A). The addition of GS967 (30 nM) reduced EADs and triggered activity (Figure 5B). The addition of GS967 (100 nM) eliminated all EADs and triggered activity and depressed spontaneous firing of PFs (Figure 5C). Figure 5D illustrates the incidence of EAD-induced triggered activity in 5 PFs exposed to ATX-II (10 nM) alone or in combination with 30 or 100 nM GS967. The incidences of EAD-induced triggered activity were 4 of 4 (100%), 1 of 4 (25%), and 0 of 5 (0%) PFs following ATX-II, 30 nM GS967, and 100 nM G967, respectively (P = .048, ATX-II vs 30 nM GS967; P = .008, ATX-II vs 100 nM GS967).

Effects of GS967 on DADs and DAD-induced triggered activity in canine PFs

Figure 6 shows a representative example of the effect of GS967 on DADs and DAD-induced triggered activity in a canine PF. High calcium (5.4 mM) and isoproterenol (1 μM) induced prominent DADs and DAD-induced triggered activity following 20 beats at BCLs of 1000, 800, 500, and 300 ms (Figure 6A). The addition of GS967 (30 nM) markedly reduced DADs and triggered activity (Figure 6B). A higher concentration of GS967 (100 nM) abolished all DADs and triggered activity (Figure 6C). Figure 6D illustrates the incidence of DAD-induced triggered activity in 4 PFs exposed to high calcium (5.4 mM), isoproterenol (1 μM), or their combination with 30 or 100 nM GS4967. The incidences of DAD-induced triggered activity were 4 of 4 (100%), 2 of 4 (50%), and 0 of 5 (0%) PFs following high calcium + isoproterenol, 30 nM GS967, and 100 nM GS967, respectively.

Effects of GS967 on DADs and DAD-induced triggered activity induced in PV and SVC sleeve preparations

We also studied the effects of GS967 on DADs and DAD-induced triggered activity elicited by exposure of PV and SVC sleeve preparations exposed to high calcium and isoproterenol. As shown in Figures 7 and 8, GS967 suppressed DADs and DAD-induced triggered activity elicited in PV (Figure 7) and SVC (Figure 8) sleeve preparations. Figure 7D illustrates the incidence of DAD-induced triggered activity in 4 PV sleeve preparations exposed to high calcium (5.4 mM), isoproterenol (1 μM), or their combination with 30 or 100 nM GS967. The incidences of DAD-induced triggered activity were 4 of 4 (100%), 1 of 4 (25%), and 0

of 4 (0%) following high calcium + isoproterenol, 30 nM GS967, and +100 nM GS967, respectively.

Discussion

Our data demonstrate that GS967, at concentrations of 30–100 nM, concentration-dependently reduces APD without altering V_{max}. In addition, GS967 was effective in (1) fully suppressing ATX-II and E-4031-induced EADs and EAD-induced triggered activity in canine PFs, (2) partially suppressing DADs induced by exposure to high calcium or high calcium + isoproterenol, and (3) fully suppressing DAD-induced triggered activity in PFs and PV and SVC sleeve preparations. Thus, the data point to a potent antiarrhythmic effect of the late sodium channel blocker GS967 in canine PFs and PV and SVC sleeve preparations. In addition, considering the high selectivity of GS967[11] and the effects of relatively low concentrations (30 nM) of this compound, these data point to an important role for late I_{Na} in the genesis of EADs and DADs.

The present study extends observations of an antiarrhythmic effect of GS967 previously made by using isolated rabbit myocytes and a rabbit in vivo model[11] to canine PFs and PV and SVC sleeve preparations. The data suggest that the effect of GS967 to suppress EAD-induced triggered activity also applies to canine PFs. The effect of GS967 to suppress DAD-mediated extrasystoles in PV and SVC sleeve preparations has not been previously reported.

Effect of GS967 in PFs

In canine PFs isolated from the right and left ventricles, GS967 shortened the AP without altering V_{max}, APA, or MDP even at fast rates and a high dose of GS967, suggesting selective block of late I_{Na} with little or no effect on peak I_{Na} at the drug concentrations evaluated. GS967 is reported to inhibit ATX-II-induced late I_{Na} in ventricular myocytes and isolated rabbit hearts with IC_{50} values of 0.13 and 0.21 μM, respectively, with minimal inhibition (7.5% at 10 μM) of peak I_{Na} and I_{Kr} (16.7% at 10 μM).[11] Interestingly, the block of peak I_{Na} by GS967 was voltage dependent, with minimal or no use-dependent effect.

Antiarrhythmic effects of GS967 in PFs exposed to QT prolonging agents

In isolated rabbit hearts and myocytes, GS967 abbreviates the duration of the ventricular AP, does not prolong the QRS interval, prevents and suppresses proarrhythmic activity, including EAD-induced triggered activity and torsades de pointes (TdP) arrhythmias, induced by the late I_{Na} enhancer ATX-II, the I_{Kr} inhibitor E-4031, and ischemia.[11] Our data illustrating the suppression of EADs and EAD-induced triggered activity demonstrate a similar effect of GS967 in canine PFs exposed to QT prolonging agents, such as E-4031 and ATX-II, to eliminate EAD-dependent extrasystoles.

Antiarrhythmic effects of GS967 under calcium overload conditions

In addition to eliminating EAD activity, GS967 was found to markedly reduce DADs and suppress DAD-induced triggered activity in canine PFs as well as in PV and SVC canine preparations. The development of DADs and DAD-induced triggered activity has been demonstrated under calcium-loading conditions in PFs.[1,2] DADs and DAD-induced triggered activity have been reported under conditions of sympathetic simulation, parasympathetic stimulation, and calcium overload conditions in canine- and rabbit-isolated single PV myocytes,[13–15] canine PV sleeve superfused preparations,[7] coronary-perfused atrial preparations,[16] and SVC sleeve preparations.[8]

DADs are known to be caused by calcium loading-induced transient inward current (I_{Ti}).[17] The suppression of DADs and DAD-induced triggered activity by GS967 is likely due to a

reduction in intracellular calcium loading (via the sodium-calcium exchanger) secondary to a decrease in intracellular sodium activity.[18] This interpretation is consistent with a previous report by Song et al,[19] showing that in guinea pig-isolated atrial myocytes, ATX-II-induced I_{Ti} and DADs are suppressed by late I_{Na} block with agents such as ranolazine.

Late I_{Na} and cardiac arrhythmias

An increase in late I_{Na} has been shown to delay cardiac repolarization in ventricular cells, tissues, and in vivo experimental models. We demonstrate the effect of the late I_{Na} agonist ATX-II to markedly prolong APD and induce EADs and EAD-induced triggered responses in canine PFs (Figures 4 and 5). Similar results have been reported in other experimental models including multicellular canine M cell preparations, canine wedge models, rabbit isolated myocytes, and in vivo and anesthetized rabbit hearts.[10,11] Like GS967, late I_{Na} blockers such as mexiletine and ranolazine are known to be effective in reversing AP prolongation and suppressing EADs as well as TdP.[10] The late I_{Na} blocker ranolazine has also been shown to reduce dispersion of ventricular repolarization, EADs, and TdP induced by drugs that block I_{Kr}.[20,21] In a rabbit model of TdP,[22] ranolazine terminated and prevented the recurrence of TdP induced by the I_{Kr} blocker clofilium.[23] Similarly, in a canine chronic atrioventricular block model, TdP induced by the I_{Kr} blocker dofetilide was suppressed by ranolazine.[24] Ranolazine has been reported to suppress TdP in several experimental models of long QT syndrome by reducing transmural dispersion of repolarization.[25–27] Arrhythmogenic effects occurring when late I_{Na} is increased by congenital (mainly long QT syndrome) or acquired pathophysiological conditions have also been shown to be reversed or prevented by ranolazine.[19]

The observed effect of the late I_{Na} inhibitor GS967 to suppress EADs (Figures 2 and 3) and TdP arrhythmias[11] when repolarization reserve is reduced secondary to I_{Kr} block[28] can be explained by the inhibition of intrinsic late I_{Na} present under physiological conditions. The inhibition of late I_{Na} is expected to be even more effective in cases in which late I_{Na} is augmented as a result of pathophysiological remodeling.

In addition to prolonging APD and inducing repolarization abnormalities, enhanced late I_{Na} increases Na influx and, via the sodium-calcium exchanger, increases intracellular calcium, resulting in calcium overload, which in turn promotes the spontaneous release of calcium from the sarcoplasmic reticulum, induction of I_{Ti}, DADs, and triggered activity. Increases in late I_{Na} also induce intracellular acidosis and activation of calmodulin-dependent protein kinase II in rat ventricular myocytes.[29] Low concentrations of GS967 are reported to reduce ATX-II-induced late I_{Na} and reverse the sodium-dependent calcium overload in rabbit myocytes.[11] The inhibition of late I_{Na} has also been shown to decrease spontaneous diastolic depolarizations because of the inhibition of a slowly inactivating tetrodotoxin-sensitive sodium current in atrial myocytes[30] and rabbit sinoatrial node cells.[31]

Late I_{Na} is increased in heart failure, and inhibitors such as ranolazine have been shown to abbreviate the AP, improve contraction, and eliminate EAD- and DAD-mediated extra-systoles in this setting.[32] In ventricular myocytes from canine failing hearts, prolonged calcium transients are abbreviated and spontaneous calcium releases are suppressed by ranolazine and tetrodotoxin.[33]

Comparison with other late sodium channel blockers

The inhibition of late I_{Na} per se has no arrhythmogenic effects in normal or diseased hearts. Arrhythmogenic effects of I_{Na} inhibitors such as flecainide can be ascribed to their effects to cause marked use-dependent reduction in peak I_{Na} and/or reduction in I_{Kr}. In contrast to flecainide, GS967 has little or no effect on fast I_{Na} or I_{Kr} at the concentrations used and

known to exert antiarrhythmic effects in experimental models. The IC_{50} for the inhibition of ATX-II-induced late I_{Na} in isolated rabbit ventricular myocytes is much greater for GS967 than for flecainide (0.13 μM vs 3.4 μM). Our data indicate that 0.1–0.3 μM is sufficient to inhibit arrhythmias induced in canine PFs and PV and SVC sleeve preparations whereas 5–10 μM of ranolazine is needed to suppress EAD-or DAD-induced triggered activity in experimental models of arrhythmias.[34,35] Thus, GS967 is a more than 10-fold more potent and more than 70-fold more selective inhibitor of late I_{Na} compared to previously described late I_{Na} inhibitors.

Conclusions

Our data indicate that the potent (IC_{50} = 130 nM) and highly selective late I_{Na} blocker GS967 exerts antiarrhythmic effects by suppressing EAD- and DAD-mediated extrasystoles in PFs and PV and SVC sleeve preparations. The data add to the growing evidence for a prominent role of late I_{Na} inhibitors as potential agents in the treatment of ventricular arrhythmias associated with long QT intervals or intracellular calcium loading.

Acknowledgments

ABBREVIATIONS

AF	atrial fibrillation
AP	action potential
APA	action potential amplitude
APD$_{90}$	action potential duration at 90% repolarization
BCL	basic cycle length
DAD	delayed afterdepolarization
EAD	early afterdepolarization
GS-458967	GS967
I$_{Kr}$	rapidly activating delayed rectifier potassium channel current
I$_{Na}$	sodium channel current
I$_{Ti}$	transient inward current
MDP	maximum diastolic potential
PF	Purkinje fiber
PV	pulmonary vein
SVC	superior vena cava
TdP	torsades de pointes
V$_{max}$	maximum rate of rise of the action potential upstroke

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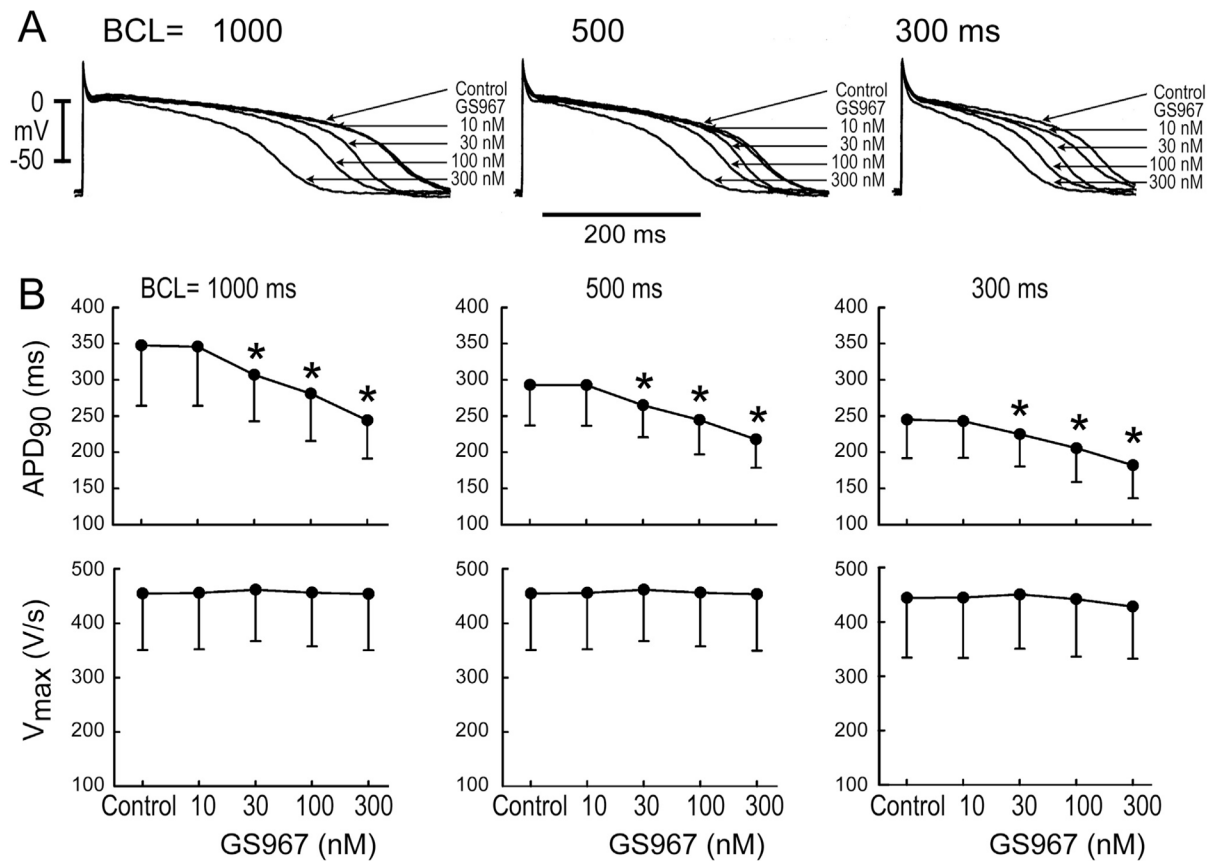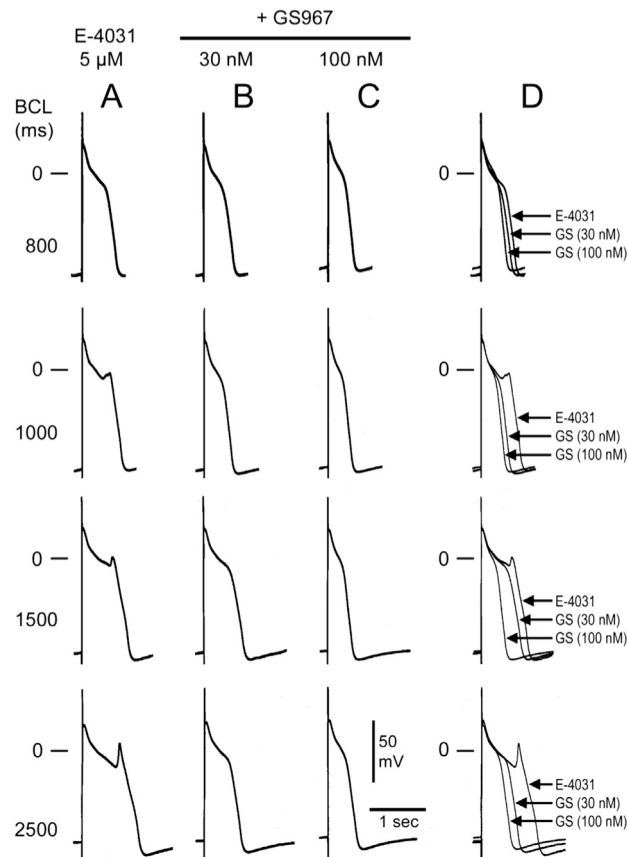
Figure 1.
Effect of GS-458967 (GS967; 10–300 nM) on action potentials (APs) recorded from canine Purkinje fibers. **A:** Effect of GS967 on AP morphology recorded at basic cycle lengths (BCLs) of 1000, 500, and 300 ms. **B:** Composite data of the effect of GS967 on the action potential duration measured at 90% repolarization (APD_{90}) and on the maximal rate of rise of the action potential upstroke (V_{max}). GS967 (10–300 nM) concentration-dependently reduced APD without reducing V_{max} (n = 5). $^{*}P < .05$ vs control.
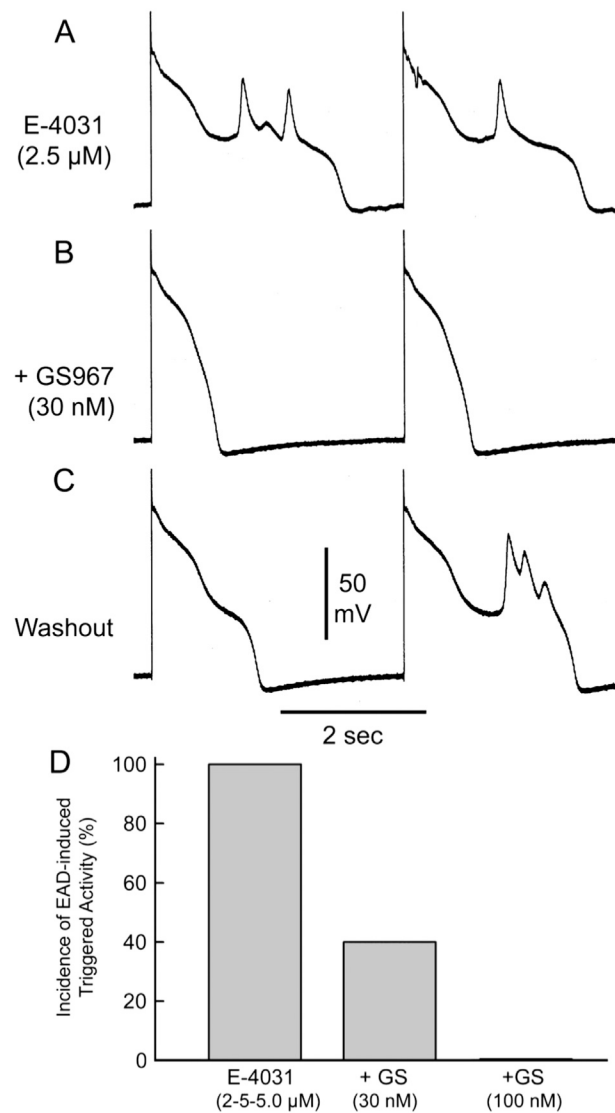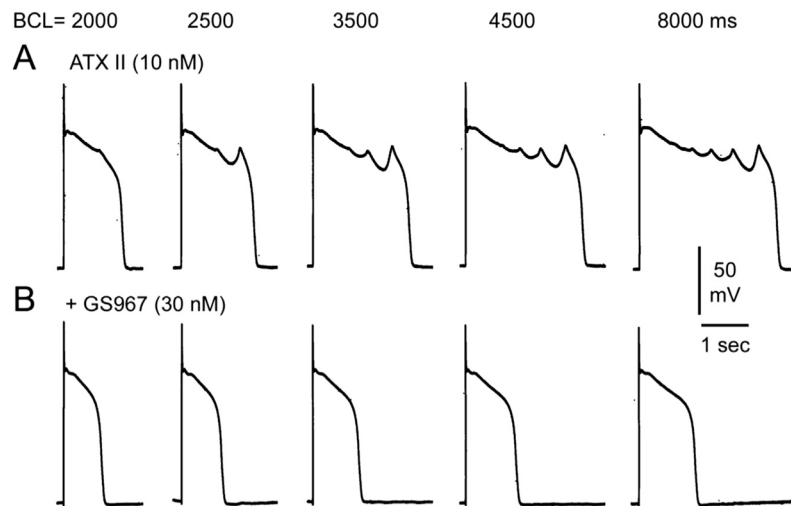
Figure 2.
GS-458967 (GS967) suppression of early afterdepolarizations (EADs) and triggered activity elicited by exposure to E-4031 in a canine Purkinje fiber. **A:** E-4031(2.5 μM) induced EADs and triggered activity. **B:** Addition of GS967 (30 nM) abolished all EADs and triggered activity. **C:** Washout of E-4031 restored EADs and triggered activity. Basic cycle length = 3500 ms. **D:** Incidence of EAD-induced triggered activity in 5 Purkinje fibers exposed to E-4031 (2.5–5 μM) alone or in combination with 30 and 100 nM GS967.
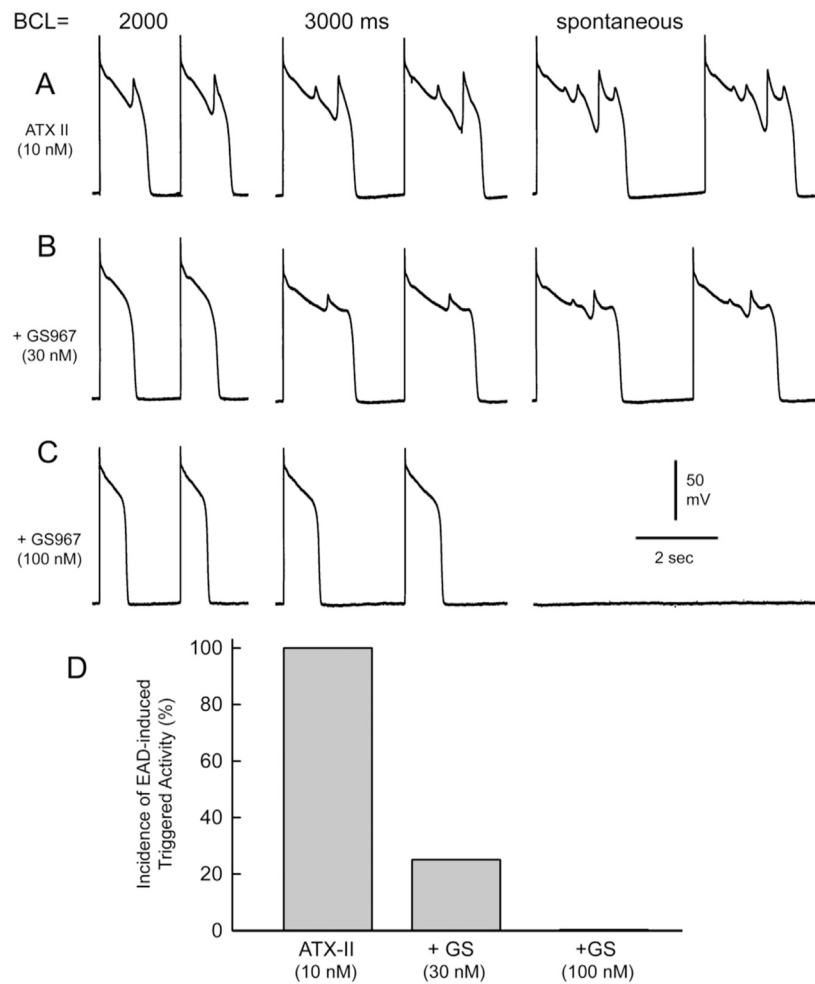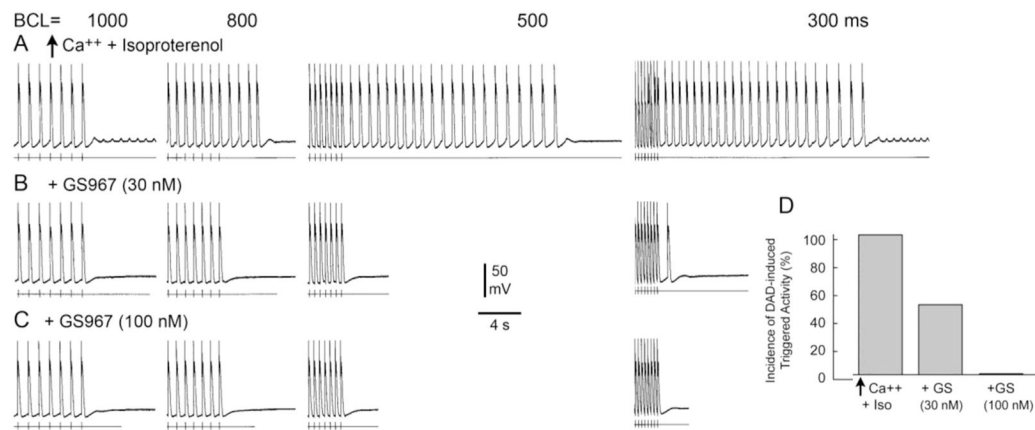
Figure 3.
Rate dependence of early afterdepolarizations (EADs) elicited by exposure to E-4031 in a canine Purkinje fiber and their inhibition by GS-967. **A:** E-4031 (5 μM) induced EADs at basic cycle lengths (BCLs) of 800, 1000, 1500, and 2500 ms. **B:** Addition of GS-458967 (GS967; 30 nM) suppressed EADs and triggered activity. **C:** Addition of GS967 (100 nM) further reduced action potential duration. **D:** Superimposed traces of the effects of E-4031 and GS967.

BCL= 2000   2500   3500   4500   8000 ms

A   ATX II (10 nM)

50 mV

1 sec

B   + GS967 (30 nM)

Figure 4.
GS-458967 (GS967) abolishes early afterdepolarizations (EADs) and EAD-induced triggered activity elicited by exposure to ATX-II in a canine Purkinje fiber. **A:** ATX-II (10 nM) elicited EADs and EAD-induced triggered activity at basic cycle lengths (BCLs) of 2000, 2500, 3500, 4500, and 8000 ms. GS967 (30 nM) abolished all EADs and triggered activity.
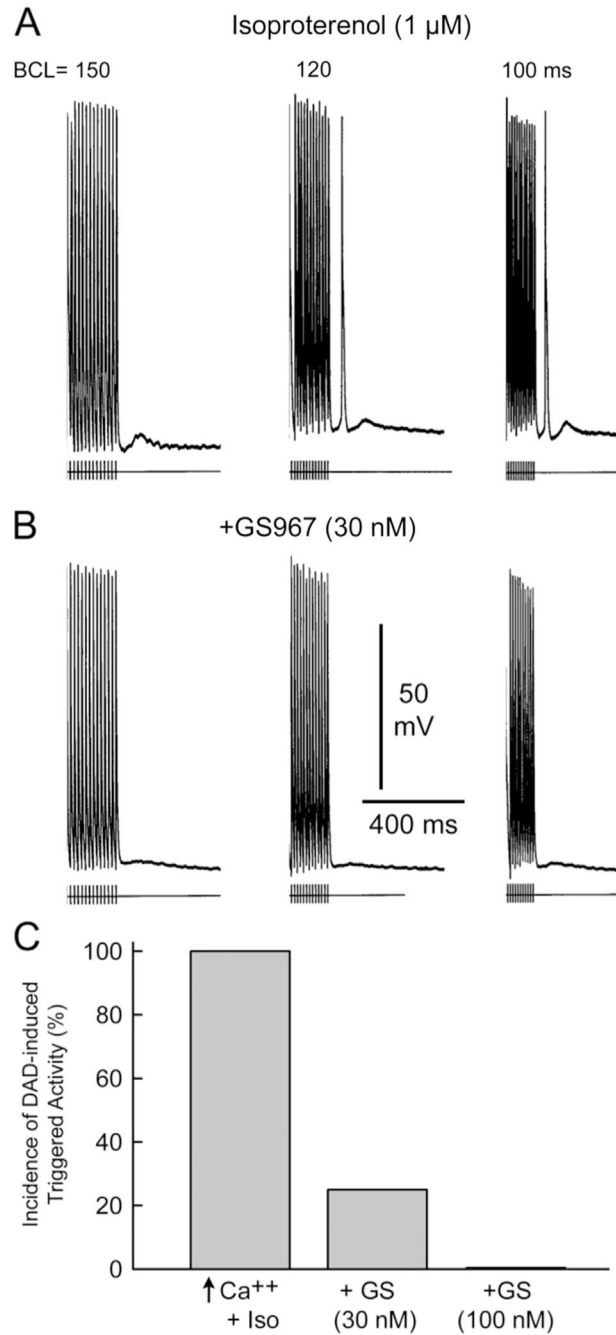
Figure 5.
Concentration and rate dependence of the effect of GS-458967 (GS967) to suppress early afterdepolarizations (EADs) and EAD-induced triggered activity elicited by exposure to ATX-II in canine Purkinje fibers. **A:** ATX-II (10 nM)-induced EADs and triggered activity at basic cycle lengths (BCLs) of 2000 and 3000 ms and during spontaneous firing. **B:** GS967 (30 nM) reduced EADs and triggered activity. **C:** GS967 (100 nM) abolished all EADs and triggered activity and depressed spontaneous firing of the Purkinje pacemaker. **D:** Incidence of EAD-induced triggered activity in 5 Purkinje fibers exposed to ATX-II (10 nM) alone or in combination with 30 and 100 nM GS967.
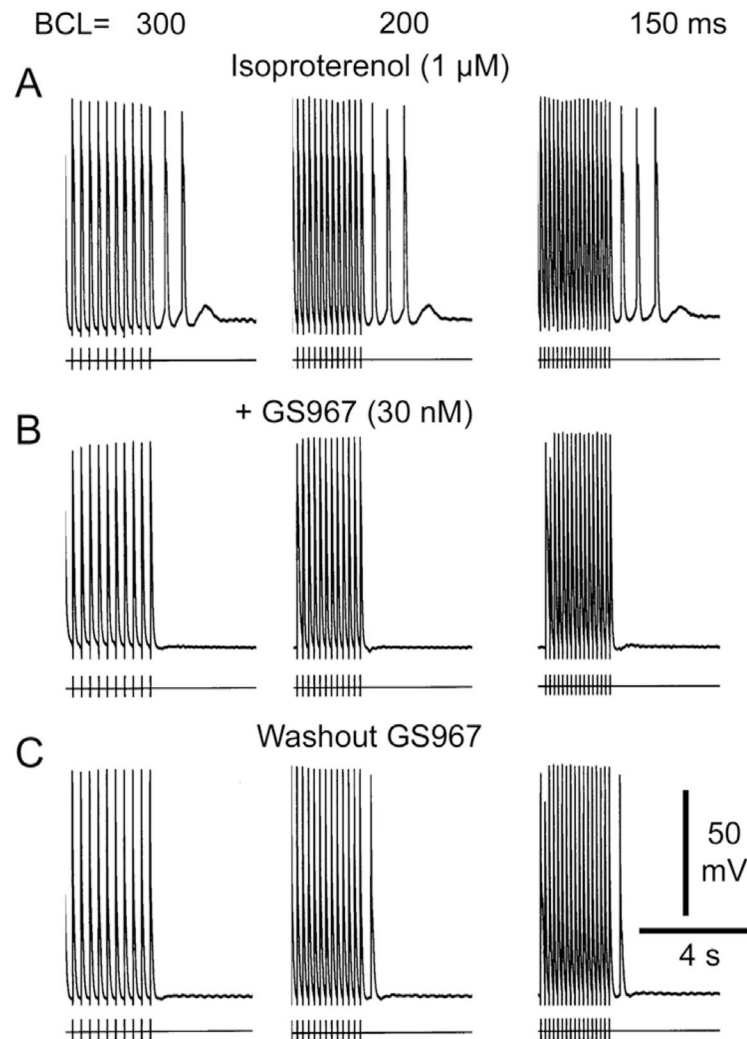
Figure 6.
Effect of GS-458967 (GS967) on delayed afterdepolarizations (DADs) and DAD-induced triggered activity elicited by exposure to high calcium and isoproterenol in a canine Purkinje fiber. **A:** High calcium (5.4 mM) and isoproterenol (1 μM) induced prominent DADs and DAD-induced triggered activity in a pulmonary vein sleeve preparation following 20 beats at basic cycle lengths (BCLs) of 1000, 800, 500, and 300 ms. **B:** GS967 (30 nM) markedly reduced DADs and triggered activity. **C:** GS967 (100 nM) completely abolished all DADs and triggered activity. **D:** Incidence of DAD-induced triggered activity in 4 Purkinje fibers exposed to high calcium (5.4 mM) + isoproterenol (l μM) alone or in combination with 30 and 100 nM GS967.

Figure 7.
GS-458967 (GS967) suppresses delayed afterdepolarizations (DADs) and DAD-induced triggered activity elicited by exposure to isoproterenol in a canine pulmonary vein (PV) sleeve preparation. **A:** Isoproterenol (1 μM) elicited a DAD following 20 beats at a basic cycle length (BCL) of 150 ms and a DAD-induced triggered response after 20 beats at BCLs of 120 or 100 ms. **B:** GS967 (30 nM) suppressed the triggered response and markedly reduced DAD activity. **C:** Incidence of DAD-induced triggered activity in 4 PV sleeve preparations exposed to high calcium (5.4 mM) + isoproterenol (1 μM) alone or in combination with 30 and 100 nM GS967.

Figure 8.
GS-458967 (GS967) suppresses delayed afterdepolarizations (DADs) and DAD-induced triggered activity elicited by exposure to isoproterenol in a canine superior vena cava sleeve preparation. **A:** Isoproterenol (1 μM) elicited DAD-induced triggered activity after 20 beats at basic cycle lengths (BCLs) of 300, 200, and 150 ms. **B:** GS967 (30 nM) abolished all triggered responses and markedly reduced DAD activity. **C:** Partial washout of the drug restored triggered activity.